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ORIGINAL ARTICLE

Elevated levels of periostin and TGF-β_1 in the bronchoalveolar lavage fluid of patients with idiopathic eosinophilic pneumonia

Shigeki Katoh,[1] Nobuhiro Matsumoto,[2] Hitomi Tanaka,[1] Naoya Yasokawa,[1] Makoto Kittaka,[1] Kouji Kurose,[1] Masaaki Abe,[1] Daisuke Yoshioka,[1] Ryo Shirai,[1] Masamitsu Nakazato,[2] Yoshihiro Kobashi[1]*

Abstract

Background: Periostin is induced in bronchial epithelial cells and fibroblasts by various stimuli including interleukin (IL)-13 and transforming growth factor (TGF)-β_1, and is involved in allergic diseases such as asthma and atopic dermatitis, playing an important role in tissue remodeling and fibrosis. The role of periostin in the pathogenesis of eosinophilic lung diseases, however, is unclear.

Objective: To examine the contribution of periostin to eosinophilic inflammation of the lung in humans, we evaluated periostin, IL-13, and TGF-β_1 levels in the bronchoalveolar lavage fluid (BALF) of patients with eosinophilic pneumonia (EP).

Methods: Periostin, IL-13, and TGF-β_1 concentrations in the BALF were measured by enzyme-linked immunosorbent assay in patients with acute EP, chronic EP, idiopathic pulmonary fibrosis (IPF), and sarcoidosis. Further, we analyzed the relationship between periostin, IL-13, and TGF-β_1, levels and the number of inflammatory cells in the BALF.

Results: The absolute number of eosinophils, and the periostin, IL-13, and TGF-β_1 levels in the BALF were significantly higher in patients with EP than in patients with IPF and sarcoidosis. Concentrations of periostin significantly correlated with the concentrations of TGF-β_1, but not those of IL-13, in the BALF of patients with EP. Periostin levels also significantly correlated with the absolute number of eosinophils in the BALF of patients with IPF, but not EP.

Conclusions: Our findings suggest that TGF-β_1 might increase the production of periostin in the lungs of patients with EP. Periostin might contribute the pathogenesis of not only EP, but also IPF.

Key words: eosinophilic pneumonia; periostin; TGF-β_1; eosinophil; idiopathic pulmonary fibrosis

From:
[1] Department of Respiratory Medicine, Kawasaki Medical School, Okayama,
[2] Neurology, Respirology, Endocrinology, and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

Corresponding author:
Shigeki Katoh
Department of Respiratory Medicine, Kawasaki Medical School
577 Matsushima, Kurashiki, Okayama 701-0192 JAPAN
Email: kshigeki@med.kawasaki-m.ac.jp

Introduction

Periostin is an extracellular matrix (ECM) protein belonging to the fasciclin family. Periostin is also reported as a matricellular protein involved in chronic allergic diseases such as asthma and atopic dermatitis, and plays an important role in tissue remodeling and fibrosis of the lung. Periostin is induced in bronchial epithelial cells and fibroblasts by various stimuli such as interleukin (IL)-13 and transforming growth factor (TGF)-β_1, binds to cellular receptors such as integrins, and activates cells.[1-3] Johansson et al reported that periostin increases the adhesion of IL-5 stimulated eosinophils by αMβ2 integrin.[4] Recently, Noguchi et al reported that periostin upregulates effector functions of eosinophils such as degranulation, and the production of cytokines such as TGF-β_1.[5]

Idiopathic eosinophilic pneumonia (EP) is an inflammatory diffuse pulmonary disease with unknown etiology characterized by the infiltration of eosinophils into the alveolar space

and interstitium of the lung.[6] Idiopathic EP includes two clinical types, acute eosinophilic pneumonia (AEP) and chronic eosinophilic pneumonia (CEP).[7,8] We previously reported that local production of IL-5 and IL-13 might be important in the pathogenesis of EP.[9,10]

Studies of periostin-deficient mice with allergic airway inflammation have produced controversial results. Periostin-deficient mice respond to lung challenge with significantly decreased numbers of eosinophils in the lung. Some studies demonstrated that mice lacking periostin exhibited increased airway resistance and mucus production, and decreased TGF-β production.[11,12] The role of periostin in the development of allergic inflammation is not clear. In humans with asthma, high levels of serum periostin are associated with high numbers of sputum and tissue eosinophils.[13] Mouse and human studies indicate that periostin might modulate allergic inflammation and could possibly have a protective role.[14]

In the present study, we evaluated the periostin, IL-13, and TGF-β_1 levels in the BALF of patients with EP, including AEP and CEP, compared with patients with sarcoidosis and idiopathic pulmonary fibrosis (IPF). Further, we analyzed the relationship between periostin, IL-13, and TGF-β_1, levels, and the number of inflammatory cells in the BALF to examine the contribution of periostin to the pathogenesis of eosinophilic inflammation of the lung.

Methods

Study Subjects

The characteristics of patients are summarized in **Table 1**. The study included 20 patients with AEP (3 women and 17 men; age, 39.9 ± 5.7 years), 20 patients with CEP (14 women and 6 men; age, 50.1 ± 4.3 years), 20 patients with IPF (3 women and 17 men; age, 68.4 ± 1.2 years), and 20 patients with sarcoidosis (12 women and 8 men; age, 55.3 ± 3.1 years). None of the patients in this study was treated with corticosteroids at the time of the investigation. Fourteen patients with AEP, 1 with CEP, 4 with IPF, and 2 with sarcoidosis were current smokers. Two patients with AEP, 19 with CEP, 6 with IPF, and 8 with sarcoidosis were never smokers. Briefly, the diagnosis of AEP was based on the following criteria established by Allen et al.[7] : acute febrile illness, severe hypoxemia (partial pressure of arterial oxygen < 60 mmHg), diffuse pulmonary infiltrates on chest radiographs, eosinophilia (> 25%) in the BALF, absence of infection and previous atopic illness, a prompt and complete response to corticosteroids, and no relapse after discontinuation of corticosteroid therapy. CEP was diagnosed according to the criteria established by Marchand et al.[8] All CEP patients had respiratory symptoms usually longer than 2 weeks' duration, alveolar eosinophilia (> 25%) in the BALF or blood eosinophilia (> 1000/mm^3), pulmonary infiltrates with a usually peripheral predominance on chest imaging, and exclusion of any known cause of eosinophilic lung disease. IPF was diagnosed according to clinical radiologic and pathologic findings based on the American Thoracic Society-European Respiratory Society consensus classification.[15] All IPF patients recruited for the current investigation exhibited the usual interstitial pneumonia pattern in high-resolution computed tomography. The diagnosis of sarcoidosis was based on examination of biopsy specimens obtained from the lung or lymph nodes showing non-caseating epithelioid cell granulomas. Smoking status had no influence on cytokine levels in the BALF of patients with each disease (data not shown). All patients provided written informed consent to participate in this study, and the human ethics review committee of University of Miyazaki and Kawasaki Medical School approved the study protocol (2054).

Table1. Characteristics of the study patients.

	AEP (n = 20)	CEP (n = 20)	IPF (n = 20)	SAR (n = 20)
Age (years)	39.9 ± 5.7[a,b]	50.1 ± 4.3	68.4 ± 1.2	55.3 ± 3.1
Sex (male/female)	17/3	6/14	17/3	8/12
Smoking status				
Current smoker	14	1	4	2
Ex-smoker	4	0	10	10
Never smoker	2	19	6	8
Total cells in BALF (× 10^5/mL)	9.1 ± 1.4[a,b]	20.3 ± 5.5[a,b]	3.5 ± 0.4	2.5 ± 0.4
Macrophages in BALF (%)	22.3 ± 2.5[a,b]	26.7 ± 4.6[a,b]	77.3 ± 4.0	71.4 ± 3.3
Lymphocytes in BALF (%)	16.1 ± 2.1	13.3 ± 3.9[a]	11.3 ± 2.0[a]	25.3 ± 3.6
Eosinophils in BALF (%)	48.6 ± 2.9[a,b]	54.7 ± 6.3[a,b]	3.2 ± 1.4	0.2 ± 0.1
Neutrophils in BALF (%)	11.2 ± 2.4[a]	4.8 ± 1.7	7.4 ±3.4	1.2 ± 0.5
Basophils in BALF (%)	1.0 ± 0.6[a,b]	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Periostin (ng/mL)	6.0 ± 1.6[a,b]	4.1 ± 1.2[a,b]	0.47 ± 0.10	0.56 ± 0.28
TGF-β_1 (pg/mL)	116.6 ± 16.4[a,b]	61.4 ± 8.3[a,b]	22.8 ± 4.2	23.9 ± 10.9
IL-13 (pg/mL)	166.1 ± 51.1[a,b]	19.8 ± 8.2[a,b]	0.22 ± 0.15	0.51 ± 0.39

AEP: Acute eosinophilic pneumonia, CEP: Chronic eosinophilic pneumonia, IPF: Idiopathic pulmonary fibrosis, SAR: Sarcoidosis, TGF: transforming growth factor, IL: interleukin. Data represent means ± SEM. [a] $P < 0.05$, compared with SAR; [b] $p < 0.05$, compared with IPF.

Bronchoalveolar Lavage

Bronchoalveolar lavage and differential cell counts were performed as described previously.[16] The supernatant of the remaining fluid was stored at -80°C until analysis. CD4+ T cells were analyzed by flow cytometry (SRL Inc, Tokyo, JAPAN).

Enzyme-Linked Immunosorbent Assay (ELISA)

Periostin levels in the BALF were measured using an ELISA kit (R&D Systems, Minneapolis, MN, USA). IL-13 levels in the BALF were measured using an ELISA kit (Abcam, Cambridge, UK). TGF-β_1 levels in the BALF were measured using an ELISA kit (BioLegend, SanDiego, CA, USA). The detection limits were 0.375 ng/mL (periostin), 0.15 pg/mL (IL-13), and 3.9 pg/ml (TGF-β_1). Concentrations below the detection limits were assumed to be zero for the purpose of statistical analysis.

Statistical Analysis

All data are expressed as mean ± standard error (SEM). The Kruskal-Wallis test was used to compare values of different groups followed by Dunn's multiple comparisons test. Spearman's correlation coefficient was used for analyses of correlations between two variables. Differences with probability values of less than 0.05 were considered significant. Statistical analysis was performed with Prism 6 software (GraphPad Software, La Jolla, CA, USA).

Results

Levels of periostin, IL-13, and TGF-β_1 in the BALF

The total number of cells and the percentage of eosinophils were significantly higher in patients with AEP and CEP than in patients with IPF and sarcoidosis (**Table 1**, $p < 0.05$). Periostin levels were significantly higher in the BALF of patients with AEP and CEP compared to patients with IPF and sarcoidosis (**Figure 1**, $p < 0.05$). To examine the contribution of IL-13 and TGF-β_1 to the production of periostin in the lungs of patients with EP, concentrations of these cytokines in the BALF were examined by ELISA. Both IL-13 and TGF-β_1 levels in the BALF were significantly higher in patients with AEP and CEP than in patients with IPF and sarcoidosis (**Figure 1**, $p < 0.05$). Next, we analyzed the relationship between the levels of periostin, IL-13, and TGF-β_1 in the BALF of patients with EP. Levels of TGF-β_1 but not IL-13, significantly correlated with the concentration of periostin in the BALF of patients with AEP ($r = 0.499$, $p = 0.025$) and CEP ($r = 0.516$, $p = 0.020$) (**Figure 2**).

Contribution of periostin to the eosinophilic inflammation of the lung

Next, to examine the contribution of periostin to the eosinophilic inflammation of the lung, we analyzed the relationship between the levels of periostin and TGF-β_1, and the numbers of inflammatory cells in the BALF of patients with EP compared

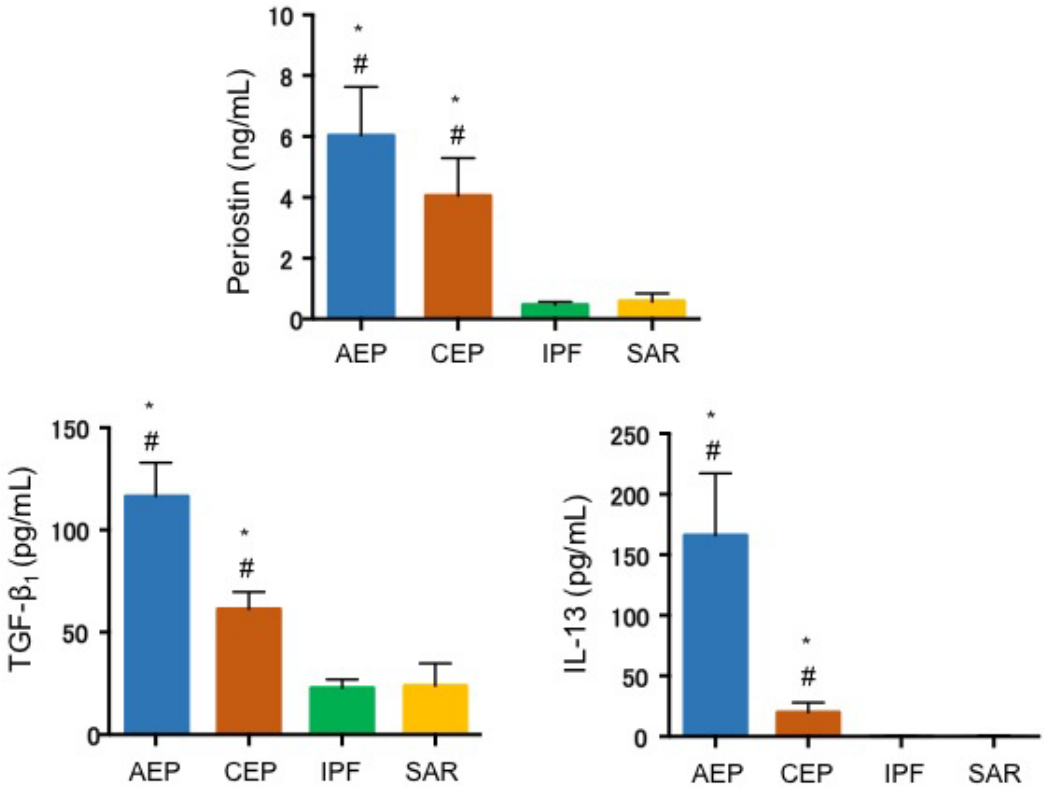

Figure 1. Concentrations of periostin, transforming growth factor (TGF)-β_1, and interleukin-13 in the bronchoalveolar lavage fluid (BALF) of patients with acute eosinophilic pneumonia (AEP), chronic eosinophilic pneumonia (CEP), idiopathic pulmonary fibrosis (IPF), or sarcoidosis (SAR). Data represent means ± SEM. *$p < 0.05$ compared with SAR; #$p < 0.05$ compared with IPF.

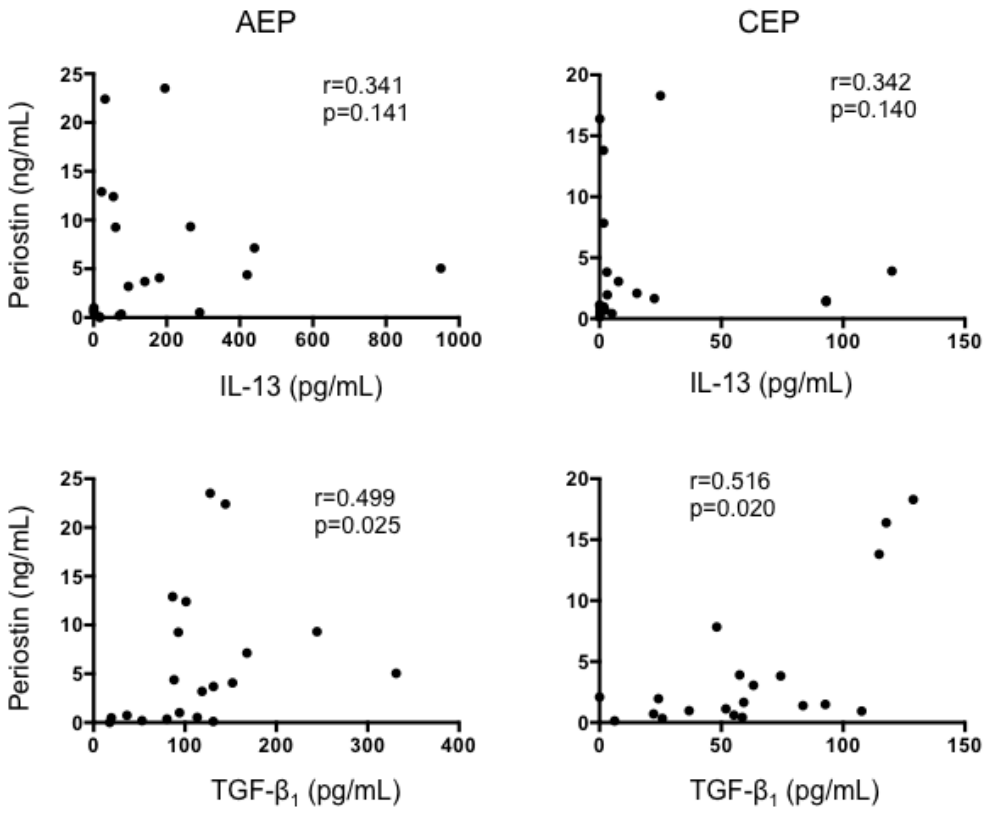

Figure 2. Relationship between periostin levels and interleukin (IL)-13 and transforming growth factor (TGF)-β_1 levels in the bronchoalveolar lavage fluid (BALF) of patients with eosinophilic pneumonia. Correlations between periostin and interleukin-13 (top), and between periostin and TGF-β_1 (bottom) concentrations in the BALF of patients with acute eosinophilic pneumonia (AEP) and those with chronic eosinophilic pneumonia (CEP) are shown.

with IPF. Interestingly, the levels of periostin significantly correlated with the absolute number of eosinophils in the BALF of patients with IPF (r = 0.487, p = 0.029), but not those with AEP (r = 0.113, p = 0.636) and CEP (r = 0.298, p = 0.202) (**Figure 3**). Furthermore, periostin levels in the BALF significantly correlated with the absolute numbers of CD4+ T cells in patients with AEP (r = 0.503, p = 0.049) and CEP (r = 0.502, p = 0.029), but not IPF (r = 0.324, p = 0.256) (**Figure 3**). TGF-β_1 levels significantly correlated with the absolute numbers of eosinophils in the BALF of patients with AEP (r = 0.540, p = 0.014), but not in those with CEP (r = 0.197, p = 0.405) and IPF (r = 0.006, p = 0.980) (**Figure 4**). TGF-β_1 levels in the BALF also significantly correlated with the absolute numbers of CD4+ T cells in patients with AEP (r = 0.632, p = 0.010) and CEP (r = 0.498, p =

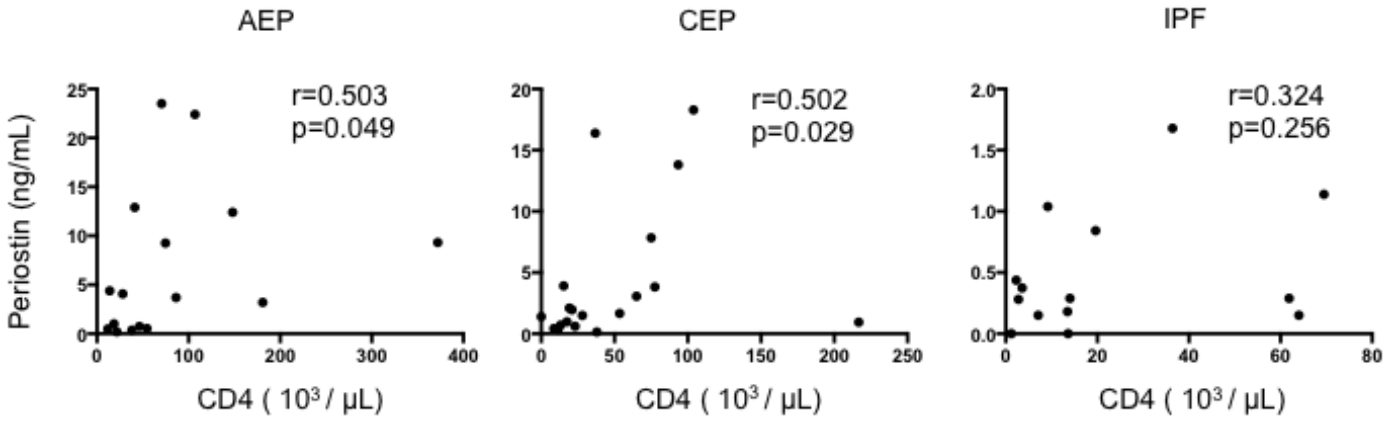

Figure 3. Relationship between the periostin levels and the number of CD4+ T cells and eosinophils in the bronchoalveolar lavage fluid (BALF) of patients with eosinophilic pneumonia and those with idiopathic pulmonary fibrosis (IPF). Correlations between the periostin concentration and the absolute number of CD4+ T cells (top), and between the periostin concentration and the absolute number of eosinophils (bottom) in the BALF of patients with acute eosinophilic pneumonia (AEP), chronic eosinophilic pneumonia (CEP), and idiopathic pulmonary fibrosis (IPF) are shown.

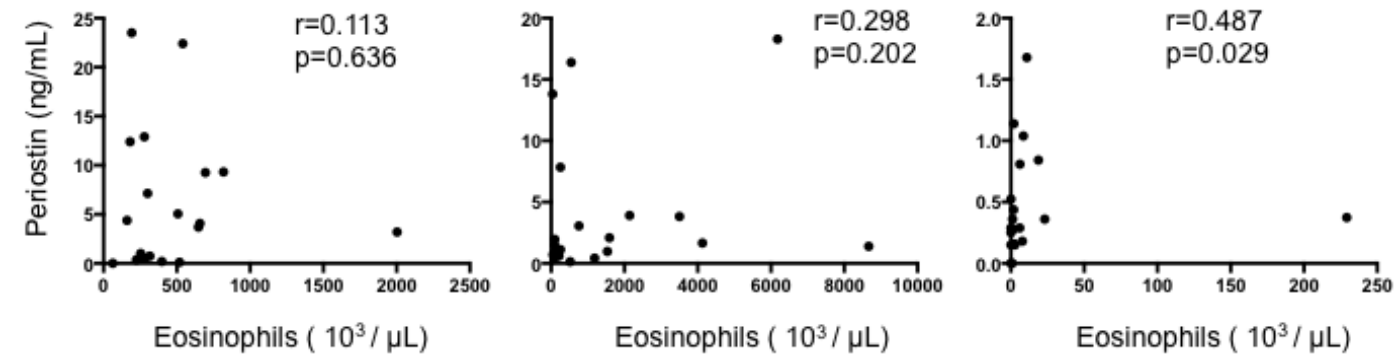

Figure 3. (Continued)

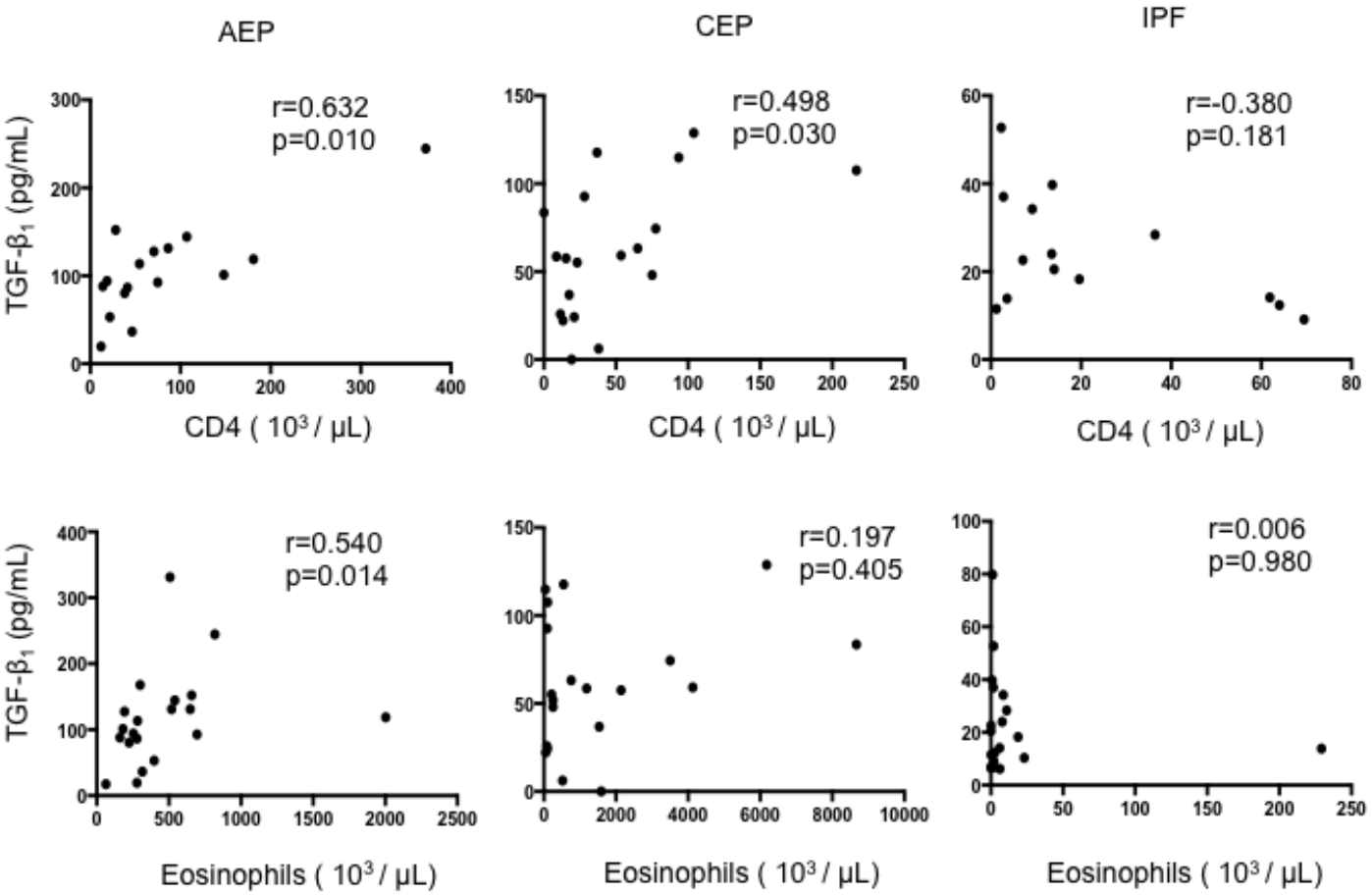

Figure 4. Relationship between the levels of transforming growth factor (TGF)-β_1 and the number of CD4+ T cells and eosinophils in the bronchoalveolar lavage fluid (BALF) of patients with eosinophilic pneumonia and those with idiopathic pulmonary fibrosis (IPF). Correlations between the TGF-β_1 concentration and the absolute number of CD4+ T cells (top), and between the TGF-β_1 concentration and the absolute number of eosinophils (bottom) in the BALF of patients with acute eosinophilic pneumonia (AEP), chronic eosinophilic pneumonia (CEP), and idiopathic pulmonary fibrosis (IPF) are shown.

0.030), but not in patients with IPF (r = -0.380, p = 0.181) (**Figure 4**). Periostin and TGF-β_1 levels did not correlate with the absolute numbers of eosinophils in the BALF of patients with sarcoidosis (data not shown).

Discussion

In the present study, we evaluated periostin, TGF-β_1, and IL-13 levels in the BALF of patients with EP compared to patients with other interstitial lung diseases, and examined the relationship between their levels and eosinophilic inflammation of the lung. Periostin, TGF-β_1, and IL-13 levels were significantly higher in the BALF of patients with EP compared to the BALF of patients with IPF and sarcoidosis. Interestingly, periostin concentrations significantly correlated with those of TGF-β_1, but not IL-13, in the BALF of patients with EP. These findings suggest that TGF-β_1, but not IL-13, plays an important role in the production of periostin in the lungs of patients with EP, although both cytokines could stimulate the production of

periostin.[1-3]

Next, we evaluated the contribution of periostin to eosinophilic inflammation of the lung in patients with EP, because recent studies demonstrated that periostin stimulates eosinophilic function.[4,5] Unexpectedly, periostin levels significantly correlated with the number of CD4+ T cells, but not with the number of eosinophils in the BALF of patients with AEP and CEP. As previously reported, Il-5 contributes to the accumulation of eosinophils in the lungs of patients with EP.[9,10] Periostin might contribute to the activation of eosinophils, but not their accumulation in the lungs of patients with EP.

Periostin promotes fibrosis and predicts disease progression in patients with IPF. Serum periostin has the potential to be a prognostic biomarker for IPF, and monomeric periostin exhibits the greatest ability to identify IPF, comparable with serum KL-6 and SP-D levels. Both monomeric and total periostin well correlate with a decline of the %VC, and % DLCO.[1,17] In the present study, periostin levels significantly correlated with the number of eosinophils in the BALF of patients with IPF. Periostin might contribute to the pathogenesis of IPF by inducing the accumulation of eosinophils into the lung. BALF eosinophilia relates to the severity of pulmonary fibrosis induced by bleomycin in rats.[18] Further studies are required to clarify the contribution of periostin and eosinophils to the pathogenesis of IPF.

TGF-β_1 is produced by various cells in the lung, such as alveolar macrophages, regulatory T cells, eosinophils, fibroblasts, and lung epithelial cells.[19-21] TGF-β_1 levels in the BALF were increased in patients with both AEP and CEP. TGF-β_1 levels significantly correlated with the number of CD4+ T cells in the BALF of both AEP and CEP patients, but TGF-β_1 levels significantly correlated with the number of eosinophils in the BALF of only patients with AEP, and not those with CEP. These findings suggest that TGF-β_1 is produced by eosinophils and CD4+ T cells in patients with AEP, while it was mainly produced by CD4+ T cells in patients with CEP. TGF-β_1 functions as both a pro-fibrotic cytokine and an immunosuppressive factor. TGF-β_1 produced by CD4+ regulatory T cells can downregulate both Th1- and Th2-type responses, and prevents eosinophilic lung disease.[22] Recently, we also demonstrated possible regulatory role of TGF-β_1 produced by CD4+CD25+T cells in the eosinophilic airway inflammation of a mouse model of chronic asthma.[23] Elevated levels of TGF-β_1 in the BALF of EP might regulate excessive Th2-type responses in patients with EP. Further studies are required to clarify the role of TGF-β_1 produced by CD4+ regulatory T cells and eosinophils in the eosinophilic inflammation of patients with EP.

Conclusion

In the present study, we demonstrated that while BALF periostin levels are increased, the levels do not correlate with the number of eosinophils in the BALF in patients with EP. Periostin might not contribute to the accumulation of eosinophils into the lung in patients with EP. The findings of the present study suggest that TGF-β_1 mainly contribute to the production of periostin in the lungs of patients with EP. Additionally, periostin might contribute to the pathogenesis of not only EP, but also IPF. Further studies are needed to confirm our findings.

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Disclosure Statement

The authors declare that they have no conflicts of interest with respect to the data presented in this paper.

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